[(Cp-R)M(CO)3] (M = Re or 99mTc) conjugates for theranostic receptor targeting

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Abstract: Cyclopentadienyl complexes of 99mTc became accessible via a retro Diels-Alder synthetic approach of dimerized cyclopentadiene derivatives. So far, this approach was limited to derivatives comprising a carboxylic acid group, directly conjugated to the Cp-ring, leading to complexes [(C5H5COOH)99mTc(CO)3] and [(C5H5CONH-R)99mTc(CO)3], respectively. The introduction of an -NCO group via Curtius rearrangement and subsequent in situ reactions with alcohols or amines gave [(C5H5NHCO-OR)2] and [(C5H5NHCO-NHR)2]. To increase the spacer lengths between the Cp-ring and the functional groups, methylene and ethylene spacers were introduced to yield C5H5-CH2COOH and C5H5-C2H4COOH respectively. The latter Cp-derivatives reacted with [99mTcO4)]- and in the presence of CO releasing/reducing agents to the corresponding [(C5H5-spacer-COOH)99mTc(CO)3] complexes. The carboxylato groups can be derivatized with targeting functions, leading to structurally altered receptor binding complexes, with 99mTc for imaging and with rhenium for therapy. The nature of the 99mTc complexes was assessed by HPLC comparison with the corresponding rhenium compounds.

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[(Cp-R)M(CO)$_3$] (M= Re or ^{99m}Tc) Conjugates for Theranostic Receptor Targeting

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[§]SCS-DSM Award for best poster presentation

Abstract: Cyclopentadienyl complexes of ^{99m}Tc became accessible *via* a retro Diels-Alder synthetic approach of dimerized cyclopentadiene derivatives. So far, this approach was limited to derivatives comprising a carboxylic acid group, directly conjugated to the Cp-ring, leading to complexes [(C_5H_5COOH)^{99m}Tc(CO)$_3$] and [(C_5H_5CONH-R)^{99m}Tc(CO)$_3$], respectively. The introduction of an –NCO group *via* Curtius rearrangement and subsequent *in situ* reactions with alcohols or amines gave [(C_5H_5NHCO-OR)$_2$] and [(C_5H_5NHCO-NHR)$_2$]. To increase the spacer lengths between the Cp-ring and the functional groups, methylene and ethylene spacers were introduced to yield C_5H_5-CH_2COOH and C_5H_5-C_2H_4COOH respectively. The latter Cp-derivatives reacted with [$^{99m}TcO_4$)]$^-$ and in the presence of CO releasing/reducing agents to the corresponding [(C_5H_5-spacer-COOH)^{99m}Tc(CO)$_3$] complexes. The carboxylato groups can be derivatized with targeting functions, leading to structurally altered receptor binding complexes, with ^{99m}Tc for imaging and with rhenium for therapy. The nature of the ^{99m}Tc complexes was assessed by HPLC comparison with the corresponding rhenium compounds.

Keywords: Bioorganometallic chemistry · Carbonic anhydrase inhibitors · Radiopharmaceuticals · Technetium · Theranostic

Introduction

Organometallic complexes with bioactive ligands are of interest for noninvasive imaging of biological events and therapeutic treatment of diseases. While several d-element cations are studied and used for therapy, ^{99m}Tc is the most prominent in nuclear medical diagnostics. It would be desirable in a *theranostic* sense (therapy and diagnostics), to have identical homologous compounds for combined therapy and diagnosis.[1–3] Rhenium and technetium belong to the same traid; therefore it is possible to use respective congener compounds for matched therapy and imaging.[4–6] While Re-based compounds can be used for therapy, the ^{99m}Tc homologs can serve as imaging agents for Single Photon Emission Computed Tomography (SPECT).[1,2,7] [(CP-R)M(CO)$_3$]-type compounds are very stable under physiological conditions and can synthetically be treated like aromatic organic molecules. Focussing on the synthesis of ^{99m}Tc-labelled radiopharmaceuticals and the corresponding cold rhenium compounds, a variety of organic and inorganic cyclopentadienyl derivatives has been synthesized and their complexes biologically investigated.

Medicinal inorganic chemistry has progressed tremendously since the successful introduction of Cisplatin in the late 1960s. During the past 50 years, a multitude of metal-containing anticancer, anti-inflammatory, antomicrobial, antimalarial and antibacterial compounds, enzyme inhibitors and MRI-contrast agents have been exploited and investigated.[8,9] The vast majority of clinically applied pharmaceuticals are of organic nature. Many of the organometallic or inorganic drug candidates are believed to act in the same way as Cisplatin whereas metal complexes for structural recognition of receptors are rarely found. Only recently, organometallic compounds came into the scope of such applications. The structural complexity of metal-containing compounds that are stable in biological systems was recognized to have an underestimated and unexplored potential for medicinal applications.[9–13]

Since nature has developed an enormous realm of functionally and structurally similar receptors and enzymes, selectivity for a specific target is a key criterion for the quality of an inhibitor.[14] Meggers and coworkers showed recently that high selectivity not only depends on intermolecular interactions but also on a versatile and directed three-dimensional arrangement of different functionalities. As exemplified by organometallic protein kinase inhibitors,[15–17] chemically inert organometallic complexes offer the opportunity to occupy biologically relevant chemical space as compared to similar inhibitors with a purely organic scaffold. Therefore, bioorganometallic complexes exhibit high potential as chemical probes.[15,18–21]

The introduction of sterically demanding metal complexes into small molecules without affecting their biological activity is challenging. One possible strategy was pioneered by Jaouen and coworkers. In the late 1970s, Hanzlik *et al.* replaced a phenyl ring in phenylalanine by ferrocene and observed that this organometallic Cp^- sandwich-complex was accepted as a substrate in the binding pocket of phenylalanine decarboxylase.[22] Based on this observation, Jaouen developed a variety of highly potent estrogen receptor inhibitors by replacing a phenyl ring in Tamoxifen with [(Cp-R)Re(CO)$_3$]. The resulting molecules retained a high binding affinity for the estrogen receptor.[12,23–28]

Combining the observations made by Meggers and by Jaouen, we studied the

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biological activity and the selectivity of [(Cp-R)Re(CO)$_3$]-complexes with different targeting vectors. In order to follow the theranostic concept, we prepared the corresponding ^{99m}Tc-complexes in water. For future projects, we investigated different Cp-building blocks comprising different functional groups bound to the Cp-ring.

Results and Discussion

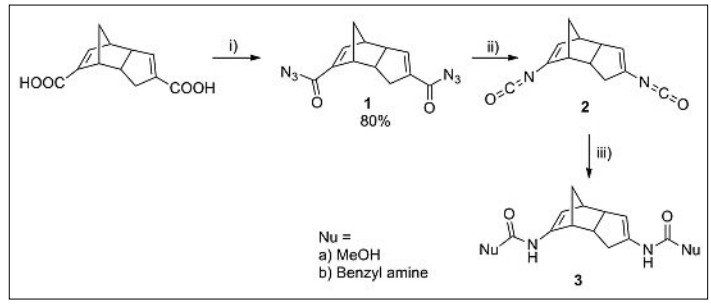
The derivatization of cyclopentadiene (HCp) or its complexes has a long history in organic and organometallic chemistry. Many different substituents have been described in literature.[29–35] However, reports for coupling biologically active targeting vectors are comparably scarce. Being part of the spacer between the chelator and the biovector, the functional group attached to Cp is of great importance for a bifunctional chelator (BFC).

We reported the unexpected formation of [(CpCOOH)^{99m}Tc(CO)$_3$] from 'Thiele's acid', the Diels-Alder dimer of HCp-COOH,[36–38] and subsequently a general aqueous synthetic route to [(Cp-R)^{99m}Tc(CO)$_3$]-type complexes was developed by derivatization of the acid function *via* amide coupling to biotargeting vectors. This procedure, where the carbonyl group is assumed to play the important role of a metal-anchoring group, allows a fully aqueous preparation of a variety of bioactive compounds.[6,37,39]

Following this approach, we recently reported organometallic carbonic anhydrase inhibitors (CAI) with superior selectivity for the pharmaceutically relevant isoforms IX and XII.[6] One of the described examples consisted of a [(CpNHCO-R)Re(CO)$_3$] building block derivatized with an arylsulfonamide targeting vector and showed the most pronounced selectivity profile. The corresponding ^{99m}Tc-complex however, has not yet been described, as the required compound HCp-NH$_2$ is still unknown.

Besides CAIs, many pharmacological active species and natural substances are aniline-based and contain the amino group in the form of amides or as internal arylsulfonamides. The replacement of such motives by the organometallic congener [(CpNHCO-R)M(CO)$_3$] (M = Re or ^{99m}Tc) would therefore increase application opportunities in therapy and in diagnosis.

Coupling of a hypothetical HCp-NH$_2$ to carboxylic acids would yield a carbonyl group close to the HCp-ring. As shown with ketones[36] and carboxylic acids,[37] the carbonyl group in α-position to the HCp is an anchoring group, binding to [^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ and initializing the *retro* Diels-Alder reaction and formation of η^5-coordinated Cp$^-$-complexes. With HCpNHCO-R, the carbonyl group would be in the β-position, still close enough to act as an anchoring group.



Scheme 1. Synthetic conditions: i) diphenylphosphoryl azide (DPPA), NEt$_3$, toluene 80%; ii) heated to 80 °C, toluene; iii) addition of the nucleophiles a/b, toluene.

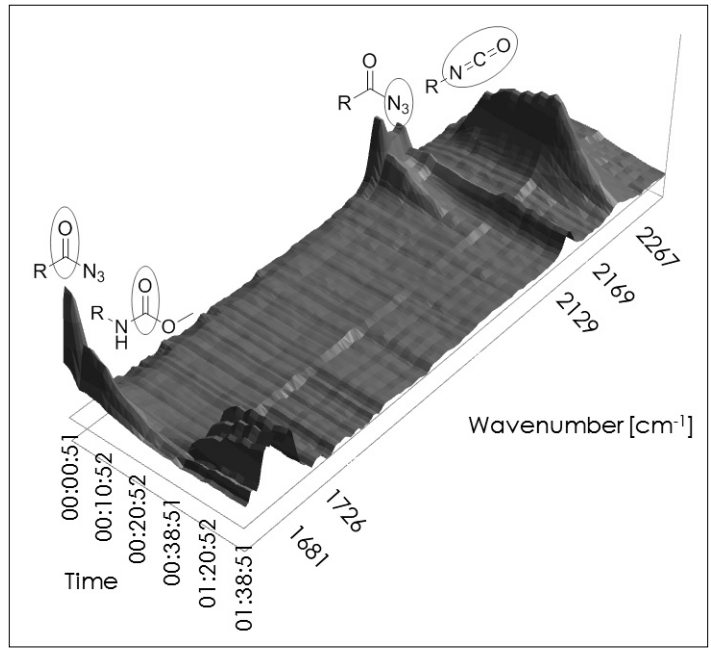
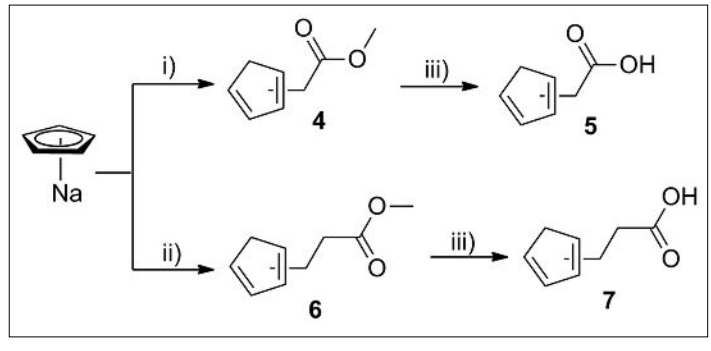
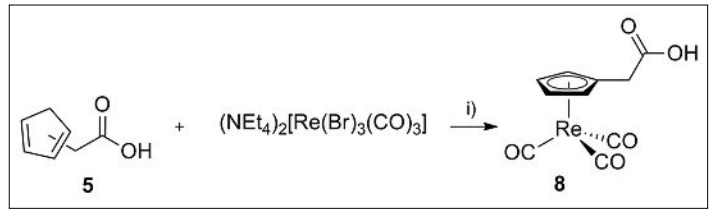


Fig. 1. Time-resolved IR-measurement of the Curtius rearrangement of **1** to **2** (complete after 20.52 min) and the following nucleophilic addition of MeOH (after 38.51 min) to form **3a**.



Scheme 2. Synthetic conditions: i) bromoacetic acid, THF, 30–50%; ii) methyl-3-brompropionate, THF, 30–50%; iii) LiOH, THF/MeOH/H$_2$O 69–74%.



Scheme 3. Synthetic conditions: i) $Na_2B_4O_7$*10H$_2$O (Borax), H$_2$O, 95 °C, 20%.

Aiming at the synthesis of HCp-NH$_2$, compounds **3a** and **3b** were synthesized by Curtius rearrangement (Scheme 1). The acid-azide **1** was obtained after treatment of Thiele's acid with diphenylphosphoryl azide in toluene. Heating to 80 °C induced the Curtius rearrangement. The resulting isocyanate reacted *in situ* with an alcohol or an amine, to form **3a** or **3b** respectively. With MeOH, the reaction was followed in a time-resolved IR-experiment, evidencing the successful transformation (Fig. 1).

Another motive in bioactive pharmaceuticals is Ar-(CH$_2$)$_n$-CONHR. Organometallic analogues of this moiety could represent biomimetics for new pharmaceuticals or imaging agents.

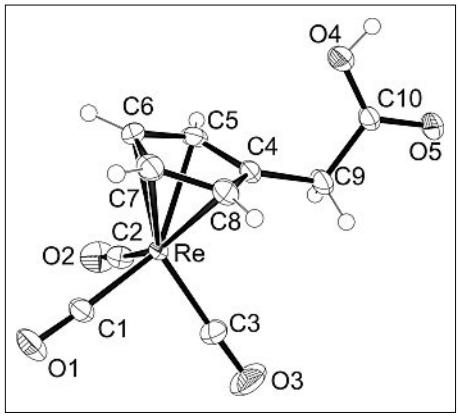


Fig. 2. ORTEP representation of **8**. Relevant bond lengths (Å): Re(1)–C(1) 1.912(2), Re(1)–C(2) 1.917(2), Re(1)–C(3) 1.913(2), Re(1)–C(7) 2.299(2), Re(1)–C(6) 2.3002(19), Re(1)–C(5) 2.301(2), Re(1)–C(4) 2.3053(19), Re(1)–C(8) 2.308(2).

As aforementioned, HCp-compounds of the form C_5H_5-COOH can be labelled as monomers or as dimers with ^{99m}Tc.[36–38] In the ligands **5** and **7** with an extended spacer, this anchoring group is retained. The carbonyl group is now in β- or in γ-position relative to the HCp-ring, likely still close enough to serve as an anchoring group. Like Thiele's acid, **5** and **7** can be derivatized with bioactive functions.

Compounds **4** and **6** have both been described before.[30,40] With bromoacetic acid methylester or bromopropionic acid methylester respectively, **4** and **6** were synthesized and basic hydrolysis gave **5** and **7** (Scheme 2).

The Re-complex **8** was synthesized directly in water. Similar to ^{99m}Tc-labeling of HCp-compounds, ligand **5** was reacted with $[ReBr_3(CO)_3]^{2-}$ in water with Borax ($Na_2B_4O_7$*$10H_2O$) at 95 °C, representing one of the rare examples of an aqueous synthesis of a complex $[(CpR)Re(CO)_3]$ (Scheme 3). Yields were low (20%) due to a competing cluster formation reaction

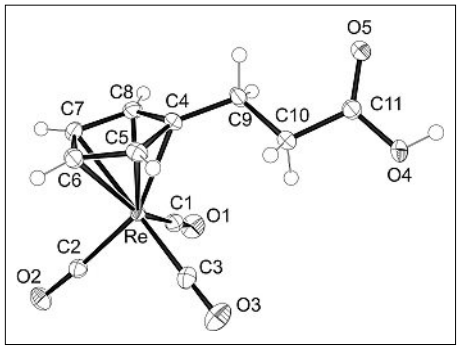
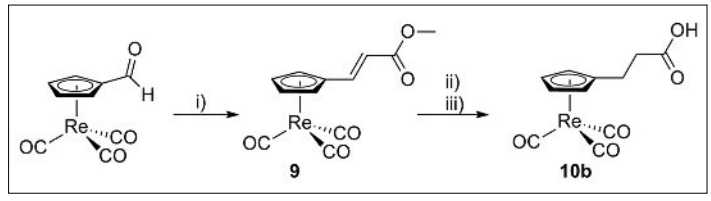


Fig. 3. ORTEP representation of **10b**. Relevant bond lengths (Å) and angles (°): Re(1)–C(2) 1.910(3), Re(1)–C(3) 1.919(3), Re(1)–C(1) 1.919(3), Re(1)–C(6) 2.287(3), Re(1)–C(5) 2.291(3), Re(1)–C(7) 2.304(3), Re(1)–C(8) 2.311(3), Re(1)–C(4) 2.324(2), C(2)-Re(1)-C(1) 90.10(12), C(3)-Re(1)-C(1) 90.61(12).



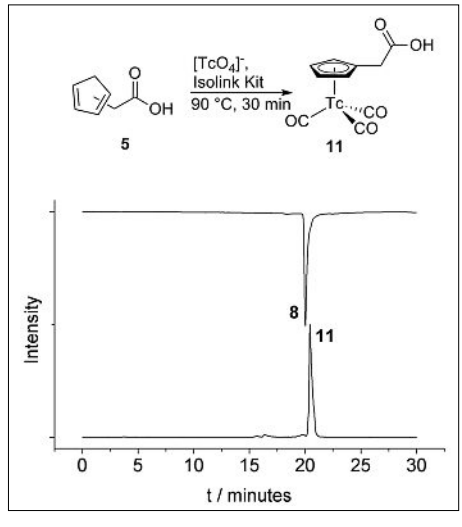
Scheme 4. Synthetic conditions: i) triethyl phosphonoacetate, MeOH, 80%; ii) H_2/Pd/C, EtOAc (**10a**). iii) LiOH, H_2O/MeOH/THF 98% (**10b**).

of $[Re(OH_2)_3(CO)_3]^+$ under basic aqueous conditions.[41]

Complex **8** was analyzed by IR (KBr), NMR ($CDCl_3$), ESI-MS (CH_3OH) and elemental analysis. Crystals were grown from CH_2Cl_2/hexane and the structure was elucidated (Fig. 2).

Carboxylic acid complex **10b** was not accessible along the same route by using ligand **7**. Instead, **10b** was obtained by Horner-Wardsworth-Emmons olefination[42] of $[(CpCOH)Re(CO)_3]$.[43] Treatment with triethyl phosphonoacetate in MeOH gave olefin **9** in 80% yield. Hydrogenation of the double bond with H_2 on Pd, followed by ester hydrolysis of **10a** with LiOH gave the desired complex **10b** (Scheme 4). Crystals were grown from CH_2Cl_2/hexane and the expected structure was confirmed (Fig. 3).

Compounds **5** and **7** were subjected to labeling studies with ^{99m}Tc. Ligands were present as monomers and in mM concentrations. The reactions were studied 'all in one'. The ligand and an Isolink Kit were sealed in a vial and $[^{99m}TcO_4]^-$ was added. Alternatively, a two-step procedure was employed; $[^{99m}Tc(OH_2)_3(CO)_3]^+$ was synthesized separately and added to the ligand. Both procedures resulted in the clean formation of the ^{99m}Tc-complexes **11** and **12** respectively (Scheme 5 and 6). No relevant amounts of side products were observed in either case. The products were analyzed by coinjection with the corresponding Re



Scheme 5. Bottom-up trace: γ-trace of the one-pot labeling of **5** with $[^{99m}TcO_4]^-$ and Isolink Kit. Top-down trace: UV-trace of Re-compound **8**. Quantitative and radiochemically pure conversion of $[^{99m}TcO_4]^-$ to complex **11**.

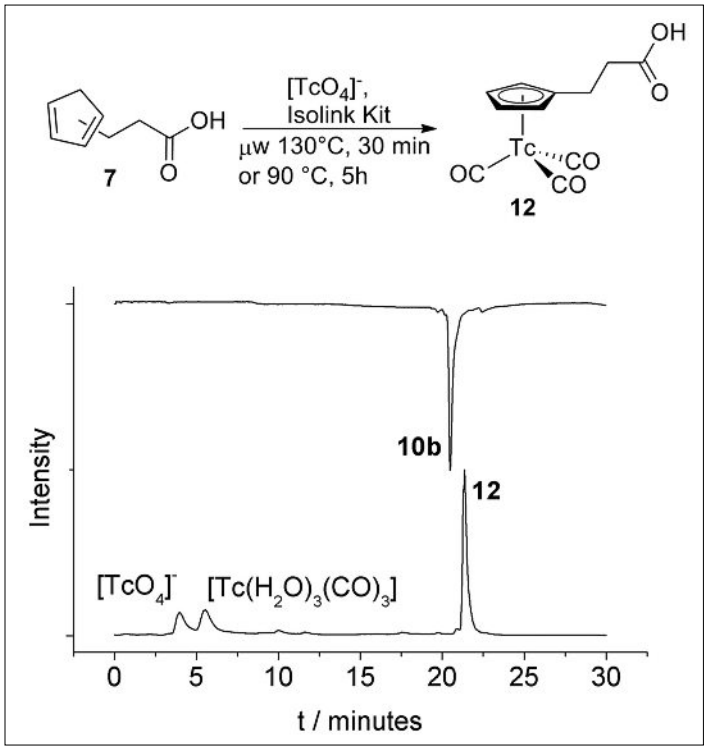
complexes. Since ^{99m}Tc complexes are present in nanomol quantities only, comparison of retention times (radio- (^{99m}Tc) and UV/vis detection (Re) are the only methods to assess the nature of ^{99m}Tc compounds. Formation rates of the ^{99m}Tc products are different for ligands **5** (hydrolyzed **4**) and **7** (hydrolyzed **6**). With **5**, formation was quantitative after 30 min (Scheme 5) whereas the reaction with **7** gave only 7% product after the same time and 62% after 5 h. Higher rates were achieved when the labeling with **7** was performed in a microwave reactor; at 130 °C and 30 min 65% product **12**, 16% $[^{99m}TcO_4]^-$ and 19% $[^{99m}Tc(OH_2)_3(CO)_3]^+$ were obtained (Scheme 6).

In order to prepare organometallic CAI with ^{99m}Tc and based on **5** and **7**,[6] compounds **13** and **14** were prepared from **8** and **10b** with rhenium. Compounds **13** and **14** extend our list of CAI, containing a methylene spacer and an ethylene spacer respectively between the carbonyl group and the Cp-ring. These methylene groups alter the distance between receptor binding motive and pendent complex and will allow a more profound insight into SARs for this important class of bioorganometallic CA targeting agents (Scheme 7).

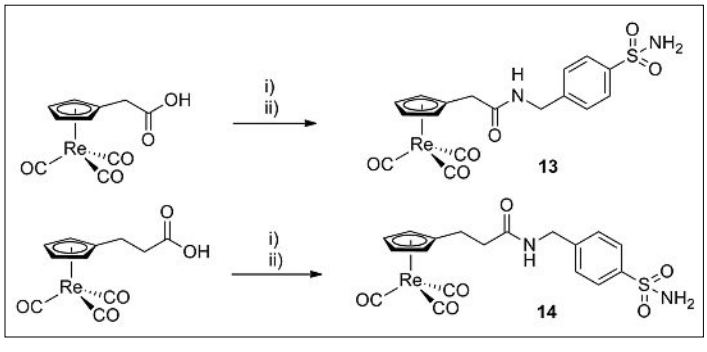
Conclusion

Derivatives of HCp comprising a CP-NHCO-R spacer were prepared in their dimeric form from Thiele's acid by use of the Curtius rearrangement. The reaction progress was investigated by time-resolved IR-measurement. Addition of nucleophiles like alcohols or amines to isocyanate **2**, generated *in situ* the compounds **3a** and **3b**.

Compounds **5** and **7** contain a carboxylic acid function in β- or in γ-position, with respect to the HCp-ring. Labelling experiments with $[^{99m}Tc(OH_2)_3(CO)_3]^+$ proved that these positions are still close enough to allow anchoring of the metal center by the carboxylic acid portion. Therefore, bringing the metal in close proximity, η^5-coordination of the HCp-portion is simplified. However, conversion rates of the ^{99m}Tc starting compounds were very different for the ligand **5** compared to **7**, which is in accordance with the increasing distance of the anchoring group to the HCp-moiety. Compound **8** was synthesized in a similar fashion to the ^{99m}Tc-compound **11** using ligand **5** with $[Re(Br)_3(CO)_3]^{2-}$ in water,



Scheme 6. Bottom-up trace: γ-trace of the one-pot labeling of **7** with $[^{99m}TcO_4]^-$ and Isolink Kit. Top-down trace: UV-trace of Re-compound **10b**.



Scheme 7. Synthetic conditions: i) pentafluorophenyl trifluoroacetate, DMF, 70–80%. ii) Mafenide, DMF, 66–78%.

representing one of the rare examples of an aqueous synthesis of a $[(CpR)Re(CO)_3]$ complex. Compound **10b** was prepared using a Horner-Wardsworth-Emmons olefination.

Compounds **13** and **14** extend our list of CAI, containing a methylene spacer and an ethylene spacer, respectively, between the carbonyl group and the Cp-ring. These groups alter the distance between receptor binding motive and pendent complex and will allow a more profound insight into SARs for this important class of bioorganometallic CA targeting agents.

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